RESPONSE OF PEA AND LENTIL TO INOCULATION WITH THE PHOSPHATE-SOLUBILIZING FUNGUS *Penicillium bilaii* (PROVIDE™)

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ABSTRACT

Researchers previously reported that inoculation with the soil fungus *Penicillium bilaii* increased dry matter production, phosphate (P) uptake, and grain yield of wheat (*Triticum aestivum*), canola (*Brassica napus, Brassica campestris*), and bean (*Phaseolus vulgaris*) by solubilizing otherwise unavailable forms of P. This study was conducted to determine if pea (*Pisum sativum*) and lentil (*Lens culinaris*) plants inoculated with *P. bilaii* are able to source otherwise unavailable forms of P and if inoculation with *P. bilaii* interferes with the activity of *Rhizobium leguminosarum* on pea and lentil. Research was conducted in the growth chamber and at 19 field locations over two years to determine the effects of inoculating pea or lentil seed with *P. bilaii* (ATCC No. 20851) or *R. leguminosarum* (USDA No. 2449), singly or in combination, on the vegetative growth, nodulation, P uptake, nitrogen (N) uptake, and grain yield of pea or lentil. Inoculation with *P. bilaii* increased total dry matter production and P uptake of pea shoots in the growth chamber experiment and of pea and lentil shoots in P-responsive field trials. Increases in P uptake following inoculation with *P. bilaii* occurred both with and without P fertilizer, were greatest at early growth stages, and were equivalent to those obtained by uninoculated plants which received greater amounts of P fertilizer. Inoculation with *P. bilaii* caused increased nodulation and N uptake of pea in the growth chamber study, and increased N uptake of pea and lentil in field studies. In 1989 field trials, all treatments interacted to increase the grain yield of pea, while in 1991 inoculation with *P. bilaii* or application of P fertilizer had little consistent effect on pea grain yield. Inoculation with *P. bilaii* increased lentil grain yield under P-responsive conditions. Inoculation with *P. bilaii* increased P availability and uptake of pea and lentil most likely by solubilizing unavailable soil P. *Penicillium bilaii* was compatible with *R. leguminosarum*. *Penicillium bilaii* is registered for use in Canada on wheat, canola, pea and lentil under the trade name PROVIDE™.

INTRODUCTION

As P plays a vital role in the nutrition of legumes, inoculation with phosphate-solubilizing (PS) microorganisms may have the potential to significantly increase legume crop productivity. Inoculation with various forms of PS bacteria increased P uptake and growth of pea (Saber et al., 1977) and bean (Khalafallah et al., 1982) in greenhouse trials. Although Badr El-Din et al. (1986) found no effect of PS bacteria on yield or nutrient uptake in soybean (*Glycine max* (L.) Merr.), Azcon-Aguilar et al. (1986) demonstrated that PS bacteria increased N concentration and uptake in soybean . Increases in nodule number, activity, N uptake, and P uptake following inoculation with PS bacteria have also been observed in alfalfa, *Medicago sativa* L. (Piccini and Azcon, 1987); and chickpea, *Cicor arietinum* L. (Alagawadi and Gaur, 1988).

Kucey (1983) identified an isolate of *Penicillium bilaii* (ATCC Strain No. 20851, previously reported as *P. bilaji*) which solubilized P from rock P at rates two to four times greater than the other isolates, grew actively on a wide range of nutrient sources, and maintained PS activity at 4°C. Inoculation with this strain of *P. bilaii* increased the vegetative growth, P uptake, or grain yield of wheat (Kucey, 1987, 1988; Asea et al., 1988; Chambers and Yeomans, 1990, 1991; Gleddie et al., 1991), bean (Kucey, 1987), and canola (Kucey and Leggett, 1989; Keyes, 1990) in growth chamber or field trials.

Few studies have examined the effects of inoculating legumes with PS microorganisms in combination with *Rhizobium leguminosarum*. It has been proposed that the organic acids produced by PS microorganisms such as *P. bilaii* may inhibit the activity of *Rhizobium* on legumes (Downey and van Kessel, 1990). Accordingly, the objective of this study was to determine the effects of inoculating pea or lentil seed with the PS fungus *P. bilaii* or *R. leguminosarum*, singly or in combination, on the vegetative growth, nodulation, P uptake, N uptake, and grain yield of pea or lentil.

MATERIALS AND METHODS

Growth Chamber

A growth chamber trial was conducted with a Brown Chernozemic soil collected from the top 0 to 15 cm of a field near Cutbank, Saskatchewan. The soil was a loamy sand which contained 13.5 mg P kg^{-1} and 29.5 mg NO_3-N kg^{-1} with a pH of 7.6. The soil was air-dried and sieved (2 mm) prior to use.

Seed of the pea cultivar Trapper was used. Four seed inoculant treatments, *P. bilaii*, *R. leguminosarum*, *P. bilaii* plus *R. leguminosarum*, and an uninoculated check were imposed over two P fertilizer regimes, O or 10 mg P kg^{-1} soil. The inoculants *P. bilaii* (ATCC Strain No. 20851) and *R. leguminosarum* (USDA No. 2449) were supplied by Philom Bios Inc.. Phosphate, where used, was applied as a suspension of $Ca(H_2PO_4)_2$ (triple super phosphate) in distilled water. Six pea seeds were planted in each 15 cm diameter pot. Pots were transferred to a Conviron PGV36/M10 growth chamber at the University of Saskatchewan and were arranged in a randomized complete block design.

Two weeks after emergence, seedlings were thinned to one plant per pot. Pots were maintained between one-half and three quarters field capacity. A micronutrient solution was applied to each pot every two weeks until harvest. Supplemental P or N was not applied.

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Each pea plant was harvested as the first flower bud opened. Days to harvest ranged from 41 to 51 days after planting. Plant shoots were harvested, dried, weighed and ground. Roots were gently washed to remove soil, placed in plastic bags, and frozen. Nodulation was rated once all the pots were harvested. Roots were gently teased apart in a shallow tray of water and nodulation per root was rated using a 1 to 10 scale. Pink to red colored nodules were considered active. The rating scale was as follows: 1, no nodules; 2, fewer than 25 inactive nodules; 3, greater than 25 inactive nodules; 4, less than 50 active nodules; 5, 50 to 99 active nodules; 6, 100 to 149 active nodules; 7, 150 to 199 active nodules; 8, 200 to 249 active nodules; 9, 250 to 299 active nodules; 10, greater than 300 active nodules. The roots were then dried, weighed, and ground. Subsamples of all ground tissue were analyzed for total N and P concentration and total N and P uptake was calculated.

Analysis of variance and single degree of freedom contrasts of the data were performed using the General Linear Models procedure of the Statistical Analysis System package (SAS Institute Inc., 1988).

Field

Field trials were conducted at 19 locations across Western Canada in 1989 and 1991. Research sites were located in the dark brown, thin black, and black soil zones, generally had low to medium levels of available soil P and N, and had never been cropped with pea or lentil.

Seed of Trapper pea or Laird lentil were used in all trials. Trials were arranged in a split-plot experimental design with five replications. Seed inoculant sub-plot treatments, *P. bilaii* or *R. leguminosarum*, applied singly or in combination, were imposed over P fertilizer main plot treatments applied as seed-placed triple super phosphate (0-45-0).

At eight pea and one lentil trial in 1991, whole-plant samples were taken at two, four, and eight weeks after emergence. Starting from the center of each plot for the two-week samples, all above-ground plant material was harvested from 1m^2 of each plot. All samples were taken from the same relative position in each plot. The four and eight-week samples were taken ever closer to the end of the plot. One-half meter border strips were left between each sample. Plant samples were dried, weighed, and ground. Subsamples of the ground tissue were analyzed for total N and P concentration, and N and P uptakes were calculated.

Prior to grain harvest the ends of each plot were trimmed to remove edge effects. The remainder of each plot was harvested with a small plot combine to measure grain yield.

As the primary objective of the field research was to evaluate the effects of inoculation of pea or lentil with *P. bilaii* over a wide range of soil and climatic conditions, only combined location analysis are presented in the Results section. Combined analysis of variance over sites was calculated as outlined by Gomez and Gomez (1984).

For the 1989 pea grain yield data all trials were combined for analysis. For the 1991 pea data, trials were seperated into P-responsive and P-nonresponsive locations based upon individual trial analysis of P uptake data. Four trials exhibited increased P uptake two weeks after emergence with the addition of P fertilizer, and four trials did not. Combined location analysis for 1991 pea dry matter production, P uptake, and N uptake for the P-responsive trials, and grain yield for P-responsive and P-nonresponsive trials, are presented in the Results section. For the 1991 lentil grain yield data, trials were seperated into P-responsive or P-nonresponsive locations based upon individual trial grain yield response to applications of P fertilizer. Combined analysis of variance and single degree of freedom contrasts of the data were performed using the General Linear Model procedure of the Statistical Analysis System package (SAS Institute Inc., 1988).

RESULTS

Phosphate application, inoculation with *R. leguminosarum*, or inoculation with *P. bilaii* increased dry matter (DM) production, total P uptake, total N uptake, and nodulation of pea in the growth chamber experiment (Table 1). Inoculation with *P. bilaii* also increased the root to shoot ratio of pea, indicating that pea roots responded to inoculation with *P. bilaii* to a greater extent than pea shoots.

Significant ($P \leq 0.01$) P by *P. bilaii* interactions occurred for DM and N uptake in the growth chamber experiment (Table 1). Inoculation with *P. bilaii* increased DM by 37% without P, but only by 11% with P, while inoculation with *P. bilaii* increased total N uptake by 35% without P, but only by 7% with P. Root DM responded to inoculation with *P. bilaii* to a greater extent than total DM, as reflected in the significant P by *P. bilaii* interaction for root to shoot ratio.

In factorial experiments, a nonsignificant interaction indicates that all significant single treatments within the interaction are additive in their effect. Thus, the nonsignificant P by *P. bilaii* by *R. leguminosarum* interactions for DM, P uptake, N uptake, and nodule rating indicate that the inclusion of each treatment increased response, and the highest level of response occurred in the presence of all three treatments (Table 1).

Phosphate application increased DM production and N uptake of pea four and eight weeks after emergence, and P uptake of pea two, four, and eight weeks after emergence over P-responsive trials established in 1991 (Table 2). Inoculation with *P. bilaii* increased DM production, P uptake, and N uptake of pea at all three sample dates except for N uptake four weeks after emergence. Inoculation with *R. leguminosarum* decreased DM production of pea at four weeks, P uptake of pea four and eight weeks, and N uptake of pea two weeks after emergence, but increased N uptake of pea eight weeks after emergence in P-responsive field trials.

Table 1. Dry matter production, root to shoot ratio, phosphorus uptake, nitrogen uptake, and nodulation of pea inoculated with *Penicillium bilaii* or *Rhizobium leguminosarum*, singly or in combination at two phosphate levels, in a growth chamber experiment.

Treatment	Total dry matter	Root:Shoot ratio	Total P uptake	Total N uptake	Nodule rating
	-- g plant^{-1} --	--	------- mg plant^{-1}	---------	-- 1 to 10 --
Check	4.71	0.46	9.6	76.1	1.4
R. leguminosarum	6.08	0.76	12.0	114.3	6.1
P. bilaii	6.96	0.98	13.3	118.0	1.7
P. bilaii + *R. leguminosarum*	7.84	0.86	14.9	139.7	8.1
[1]TSP + Check	6.80	0.82	13.5	115.1	2.1
TSP + *R. leguminosarum*	7.23	0.72	15.2	137.1	7.7
TSP + *P. bilaii*	7.63	0.85	15.9	122.7	2.1
TSP + *P. bilaii* + *R. leguminosarum*	7.93	0.75	17.4	147.4	8.1
LSD (P≤0.05)	0.67	0.15	1.8	13.4	1.2
Contrast:					
Phosphate (P) vs.	7.40**	0.77	15.5**	130.6**	5.0*
no phosphate	6.40	0.78	12.5	112.0	4.3
P. bilaii (Pb) vs.	7.59**	0.86**	15.4**	132.0**	5.0*
no *P. bilaii*	6.21	0.69	12.6	110.7	4.3
R. leguminosarum (R) vs.	7.27**	0.77	14.9**	134.6**	7.5**
no *R. leguminosarum*	6.53	0.78	13.1	108.0	1.8
Pb x R	NS	**	NS	NS	NS
P x Pb	**	**	NS	**	NS
P x R	**	*	NS	NS	NS
P x Pb x R	NS	**	NS	NS	NS

*, **, NS: Significant at P≤0.05, P≤0.01, and nonsignificant, respectively.
[1] Triple super phosphate, 10 mg P kg^{-1} soil.

Table 2. Dry matter production, phosphorus uptake, and nitrogen uptake two, four, and eight weeks after emergence at phosphate-responsive field locations[1] for pea inoculated with *Penicillium bilaii* or *Rhizobium leguminosarum*, singly or in combination, at three phosphate levels, in 1991.

P_2O_5 rate	Inoculant	Sampling date 2 wk	4 wk	8 wk	2 wk	4 wk	8 wk	2 wk	4 wk	8 wk
kg ha^{-1}		------ dry matter (g m^{-2}) ------			------ P uptake (mg m^{-2}) ------			------ N uptake (mg m^{-2}) ------		
0	Check	6.5	22.9	370	22.5	101	965	331	1100	11000
	R. leguminosarum	6.1	19.4	351	21.7	86	840	322	960	11400
	P. bilaii	7.0	23.8	381	24.9	109	1000	359	1100	11800
	P. bilaii + *R. leg.*	6.5	20.7	350	23.4	91	831	329	1050	11100
10	Check	6.0	20.6	354	22.6	97	859	305	960	10500
	R. leguminosarum	6.7	21.7	378	25.1	98	889	334	1080	12200
	P. bilaii	6.9	22.5	410	27.1	101	1013	353	1000	12500
	P. bilaii + *R. leg.*	6.7	21.7	393	25.9	98	931	338	1030	12900
20	Check	6.4	24.6	386	27.3	114	972	332	1110	11700
	R. leguminosarum	6.3	23.4	387	27.3	108	952	320	1120	13100
	P. bilaii	7.4	27.7	399	31.6	125	1037	378	1190	12300
	P. bilaii + *R. leg.*	6.6	23.4	374	28.4	108	892	334	1140	12200
LSD (P≤0.05):	Phosphate	0.3	2.1	17	1.3	9	60	16	101	660
	Inoculant	0.4	1.6	21	1.5	8	59	19	69	790
Contrast:										
Phosphate 0		6.5	21.7	363	23.1	97	909	335	1060	11300
Phosphate 10		6.6	21.6	384	25.2	99	923	333	1020	12000
Phosphate 20		6.7	24.8	387	28.7	114	963	341	1140	12300
Phosphate (P)-linear		NS	**	**	**	**	†	NS	†	**
Phosphate-quadratic		NS	†	NS	NS	†	NS	NS	†	NS
P. bilaii (Pb) vs.		6.9**	23.3*	385†	26.9**	105†	951†	349**	1090	12100†
no *P. bilaii*		6.3	22.1	371	24.4	100	913	324	1060	11700
R. leguminosarum (R) vs.		6.5	21.7**	372	25.3	98**	889**	330*	1060	12200†
no *R. leguminosarum*		6.7	23.7	383	26.0	108	974	343	1080	11600
Pb x R		†	NS	†	*	NS	*	*	NS	*
P-lin x Pb		NS	NS	NS	NS	NS	NS	NS	NS	NS
P-quad x Pb		NS	NS	*	NS	NS	*	NS	NS	*
P-lin x R		NS	NS	NS	NS	NS	NS	NS	NS	NS
P-quad x R		**	**	NS	NS	*	*	*	*	NS
P-lin x Pb x R		NS	NS	NS	NS	NS	NS	NS	NS	NS
P-quad x Pb x R		NS	NS	NS	NS	NS	NS	NS	NS	NS

†, *, **, NS: Significant at P≤0.10, P≤0.05, P≤0.01, and nonsignificant, respectively.
[1] Summary of data from field trials at four phosphate-fertilizer-responsive locations in 1991.

Significant ($P \leq 0.05$) P-quadratic by *P. bilaii* interactions occurred for DM production, P uptake, and N uptake of pea eight weeks after emergence over P-responsive field trials (Table 2). Thus, main effects were not additive, and the highest level of response occurred at the mid-rate of P, with *P. bilaii* inoculation. However, as no interactions occurred between P and *P. bilaii* two and four weeks after emergence, the effects of P and *P. bilaii* were additive, and the highest levels of DM, P uptake, and N uptake occurred when the highest rate of P was applied with *P. bilaii*.

Application of P increased DM and P uptake of lentil two weeks after emergence, while inoculation with *P. bilaii* increased DM and P uptake of lentil two and four weeks after emergence, and N uptake of lentil four weeks after emergence (Table 3). Inoculation with *R. leguminosarum* decreased DM production or N uptake of lentil two or four weeks after emergence, respectively.

As no P by *P. bilaii* interactions occurred for DM production or P uptake of lentil two weeks after emergence, their effects were additive, and the highest level of response occurred at the high-rate of P application with *P. bilaii*.

Table 3. Dry matter production, phosphorus uptake, and nitrogen uptake two, four, and eight weeks after emergence for lentil inoculated with *Penicillium bilaii* or *Rhizobium leguminosarum*, singly or in combination at three phosphate levels, in a 1991 field trial.

P_2O_5 rate	Inoculant	Sampling date								
		2 wk	4 wk	8 wk	2 wk	4 wk	8 wk	2 wk	4 wk	8 wk
kg ha^{-1}		------ dry matter (g m^{-2}) ------			------ P uptake (mg m^{-2}) ------			------ N uptake (mg m^{-2}) ------		
0	Check	16.6	25.8	251	43.5	112	891	515	1043	6820
	R. leguminosarum	15.6	21.0	247	40.4	93	910	472	860	7535
	P. bilaii	19.0	25.6	259	49.1	112	980	588	1052	6914
	P. bilaii + R. leg.	18.2	26.0	244	44.7	114	920	571	1019	6520
10	Check	19.4	25.2	245	47.5	109	921	585	1000	6211
	R. leguminosarum	15.2	22.0	262	42.7	95	980	522	867	6757
	P. bilaii	21.6	24.8	208	52.5	109	777	582	985	5932
	P. bilaii + R. leg.	17.6	25.6	247	45.9	115	896	544	1042	6275
20	Check	20.6	25.0	246	59.3	111	925	574	1024	6591
	R. leguminosarum	18.8	21.4	245	52.0	96	935	523	836	6012
	P. bilaii	21.2	23.4	265	58.4	104	1028	583	942	6843
	P. bilaii + R. leg.	22.2	25.4	236	67.1	113	886	620	988	6555
LSD ($P \leq 0.05$):	Phosphate	2.7	2.9	28	8.9	13	110	103	134	678
	Inoculant	3.1	2.7	28	8.2	12	116	101	99	945
Contrast:										
Phosphate 0		17.4	24.6	250	44.4	108	927	536	994	6948
Phosphate 10		18.5	24.4	241	47.1	107	894	558	974	6294
Phosphate 20		20.7	23.8	247	59.2	106	939	575	947	6482
Phosphate (P)-linear		*	NS	NS	**	NS	NS	NS	NS	NS
Phosphate-quadratic		NS	NS	NS	NS	NS	NS	NS	NS	NS
P. bilaii (Pb) vs.		20.0*	25.1†	243	52.9†	111*	915	581	1005†	6507
no *P. bilaii*		17.7	23.4	249	47.6	103	927	532	938	6654
R. leguminosarum (R) vs.		17.9†	23.6	247	48.8	104	921	542	935*	6609
no *R. leguminosarum*		19.7	25.0	245	51.7	109	920	571	1008	6552
Pb x R		NS	**	NS	NS	**	NS	NS	**	NS
P-lin x Pb		NS	NS	NS	NS	NS	NS	NS	NS	NS
P-quad x Pb		NS	NS	NS	NS	NS	†	NS	NS	NS
P-lin x R		NS	NS	NS	NS	NS	NS	NS	NS	NS
P-quad x R		NS	NS	*	NS	NS	NS	NS	NS	NS
P-lin x Pb x R		NS	NS	NS	NS	NS	NS	NS	NS	NS
P-quad x Pb x R		NS	NS	NS	NS	NS	NS	NS	NS	NS

†, *, **, NS: Significant at $P \leq 0.10$, $P \leq 0.05$, $P \leq 0.01$, and nonsignificant, respectively.

Application of P or inoculation with *R. leguminosarum* increased grain yield of pea over trials established in 1989 (Table 4). Inoculation with *P. bilaii* had no main effect on grain yield. However, as indicated by the significant ($P \leq 0.01$) *P. bilaii* by *R. leguminosarum* interaction, inoculation with *P. bilaii* plus *R. leguminosarum* significantly ($P \leq 0.01$) increased grain yield as compared to inoculation with *R. leguminosarum* alone.

No interactions occurred between P fertilizer and inoculant treatments for pea grain yield in 1989 indicating that significant single treatments were additive in effect (Table 4). The highest grain yield occurred when all three treatments were present.

Over P-responsive locations, application of P increased grain yield of pea in 1991 (Table 4). Inoculation with *P. bilaii* increased grain yield as compared to the check, while inoculation with *R. leguminosarum* decreased pea yield in P-responsive trials.

Application of P had no main effect on lentil grain yield over P-responsive locations, however, the grain yield of the check increased with the application of 10 kg P_2O_5 ha^{-1} (Table 4). Inoculation with *P. bilaii* or *R. leguminosarum* increased lentil grain yield over P-responsive locations. As indicated by the lack of interactions, treatments were additive in their effect and the highest lentil grain yield occurred when all three treatments were present.

Over P-nonresponsive locations, pea grain yield increased with the addition of 10 kg P_2O_5 ha^{-1}, but decreased with the addition of 20 kg P_2O_5 ha^{-1} (Table 4). Inoculation with *P. bilaii* had no effect while inoculation with *R. leguminosarum* increased grain yield of pea. Application of P had no effect on grain yield of lentil in P-nonresponsive trials, while inoculation with *P. bilaii* or *R. leguminosarum* decreased lentil grain yield.

Table 4. Grain yield at phosphate-responsive[1] and phosphate-nonresponsive[2] field locations for pea or lentil inoculated with *Penicillium bilaii* or *Rhizobium leguminosarum*, singly or in combination, at three phosphate levels.

P_2O_5 rate	Inoculant	P-responsive locations			P-nonresponsive locations	
		Pea		Lentil		
		1989	1991	1991	1991	1991
kg ha^{-1}		grain yield (kg ha^{-1})				
0	Check	2754	3166	1700	4101	2817
	R. leguminosarum	2999	2992	2416	4348	2585
	P. bilaii	2662	3165	1984	3928	2492
	P. bilaii + *R. leg.*	3197	3007	2420	4291	2450
10	Check	----	3194	1848	4183	2537
	R. leguminosarum	----	3166	2463	4350	2451
	P. bilaii	----	3441	2030	4076	2545
	P. bilaii + *R. leg.*	----	3126	2480	4389	2488
20	Check	3027	3266	1816	3932	2647
	R. leguminosarum	3135	3123	2435	4215	2563
	P. bilaii	2934	3313	1991	3963	2696
	P. bilaii + *R. leg.*	3405	3081	2524	4385	2481
LSD ($P \leq 0.05$):	Phosphate	216	118	127	110	149
	Inoculant	120	121	87	112	113
Contrast:						
Phosphate 0		2903	3083	2130	4167	2586
Phosphate 10		----	3232	2205	4249	2505
Phosphate 20		3123	3196	2192	4124	2597
Phosphate (P)-linear		*	†	NS	NS	NS
Phosphate-quadratic		----	†	NS	*	NS
P. bilaii (Pb) vs.		3048	3189	2238**	4172	2525†
no *P. bilaii*		2979	3151	2113	4188	2600
R. leguminosarum (R) vs.		3183**	3083**	2456**	4330**	2503**
no *R. leguminosarum*		2844	3257	1895	4030	2622
Pb x R		**	NS	*	†	NS
P-lin x Pb		NS	NS	NS	*	*
P-quad x Pb		----	NS	NS	NS	†
P-lin x R		NS	NS	NS	NS	NS
P-quad x R		----	NS	NS	NS	NS
P-lin x Pb x R		NS	NS	NS	NS	NS
P-quad x Pb x R		----	NS	NS	NS	NS
P. bilaii vs.		2798	3306†	2002**	3989	2578
Check		2891	3209	1788	4072	2667
P. bilaii + *R. leg.* vs.		3299**	3071	2475	4355	2473
R. leguminosarum		3067	3094	2438	4304	2533

†, *, **, NS: Significant at $P \leq 0.10$, $P \leq 0.05$, $P \leq 0.01$, and nonsignificant, respectively.
[1] Six pea trials in 1989, four pea trials and three lentil trials in 1991.
[2] Four pea and two lentil trials in 1991.

DISCUSSION

Inoculation with *P. bilaii* increased vegetative growth, P uptake, and N uptake of pea in the growth chamber study, and increased vegetative growth, P uptake, N uptake, and grain yield of pea and lentil under P-responsive field conditions. These results are in agreement with previous reports of increased yield and P uptake of wheat and canola following inoculation with *P. bilaii* (Kucey, 1987, 1988; Kucey and Leggett, 1989; Chambers and Yeomans, 1990, 1991; Keyes, 1990; and Gleddie et al, 1991). Others have also demonstrated that inoculation with *P. bilaii* resulted in increased dry matter production of pea (Downey and van Kessel, 1990), and dry matter production and P uptake of bean (Kucey, 1987) in growth chamber studies.

Increased P uptake observed in *P. bilaii*-inoculated plants is most likely due to solubilization of otherwise unavailable soil P. As tracers were not used in these studies, the source of additional P in *P. bilaii*-inoculated pea or lentil plants cannot be determined. *Penicillium bilaii* solubilizes rock P in solution culture, most likely by the production of organic acids which acidify the surrounding media (Kucey, 1983; Asea et al., 1988). Previous studies using ^{32}P indicated that plants inoculated with *P. bilaii* are able to utilize soil P sources which are unavailable to control plants (Chambers and Yeomans, 1990, 1991) as well as added rock P (Asea et al., 1988). This is consistent with the results of this study which demonstrate increased P uptake by *P. bilaii*-inoculated plants without the addition of P fertilizer.

Further indirect evidence that inoculation with *P. bilaii* increases the availability of soil P is provided by comparisons of *P. bilaii*-inoculated plants to plants which received P fertilizer alone. Increasing soil-available P by adding P fertilizer increased total plant P uptake in the growth chamber and in field studies, providing no other factor was limiting plant growth. Inoculation with *P.*

bilaii without P fertilizer resulted in equivalent P uptakes to those using 10 mg P kg^{-1} soil in the growth chamber study, and to those obtained using 10 kg P_2O_5 ha^{-1} in P-responsive field trials. Inoculation of pea with *P. bilaii* at 10 kg P_2O_5 ha^{-1} in P-responsive field trials resulted in equivalent P uptakes to those obtained using 20 kg P_2O_5 ha^{-1} at two and eight weeks after emergence. Thus, inoculation with *P. bilaii* increased soil P availability equivalent to the increases in soil P availability obtained by adding 10 mg P kg^{-1} soil in the growth chamber, or by adding 10 kg P_2O_5 ha^{-1} P fertilizer in the field studies.

Furthermore, if inoculation with *P. bilaii* does increase the availability of soil P to pea or lentil plants, grain yield response to *P. bilaii* inoculation should occur only under conditions where P fertilizer increases grain yield, as was observed in this study. Also, the greatest P uptake response to *P. bilaii* occurred early in the growing season, and *P. bilaii* and P fertilizer were generally additive in their effects on P uptake and grain yield of pea and lentil. This is consistent with the hypothesis that *P. bilaii*-inoculated plants can source otherwise unavailable forms of P.

The P-solubilizing activity of *P. bilaii* did not interfere with the activity of *R. leguminosarum*. Inoculation with *P. bilaii* resulted in increased nodulation and N uptake of pea plants in the growth chamber experiment , and promoted increased N uptake by pea and lentil plants in P-responsive field trials. The increases in nodulation and N uptake following inoculation with *P. bilaii* coincided with those caused by the addition of P fertilizer. Grain yield of pea or lentil inoculated with *R. leguminosarum* was either increased or not affected by co-inoculation with *P. bilaii*. These observations contradict Downey and van Kessel (1990) who concluded that inoculating with *P. bilaii* decreased total N accumulation by pea plants in a growth chamber experiment, and speculated that the production of P-solubilizing organic acids by *P. bilaii* may have reduced the rhizosphere pH to a degree which would inhibit *Rhizobium* function. However, in their study, the application of P fertilizer had no effect on N accumulation by *R. leguminosarum*-inoculated pea plants, and the negative effect of the *P. bilaii* treatments on N uptake was driven solely by an abnormally low dry matter yield for the *P. bilaii* plus *R. leguminosarum* treatment without applied P. If *P. bilaii* did directly inhibit the activity of *R. leguminosarum* as they hypothesized, the *P. bilaii* plus *R. leguminosarum* plus P fertilizer treatment should also be inferior as compared to the *R. leguminosarum* plus P fertilizer treatment. This was not the case. Dry matter production, total N, and percent N derived from N_2 fixation of the *P. bilaii* plus *R. leguminosarum* plus P treatment was equivalent to the *R. leguminosarum* plus P treatment. Several other researchers, working with PS bacteria known to produce organic acids, have found increased nodule numbers, weight, nitrogenase activity, and total plant N uptake when P-solubilizing bacteria were applied with *Rhizobium* (Azcon-Aguilar and Barea, 1978; Azcon-Aguilar et al., 1986; Alagawadi and Gaur, 1988). Kucey (1987) observed increased vegetative growth and P uptake of bean inoculated with both *P. bilaii* and *R. phaseoli* as compared to bean inoculated with *R. phaseoli* alone. The results of this study indicate that inoculation with *P. bilaii* does not inhibit the activity or function of *R. leguminosarum* on pea or lentil plants.

Overall, *P. bilaii* inoculation resulted in increased pea and lentil plant growth , P uptake, N uptake, and in some trials, grain yield. It appears that by solubilizing otherwise unavailable soil P, *P. bilaii* is able to increase P availability and uptake by pea and lentil. Inoculation with *P. bilaii* had no adverse effects on the nodulation of pea plants by *R. leguminosarum* or on N assimilation by pea or lentil. Increased P assimilation due to inoculation with *P. bilaii* enhanced the N_2 fixation processes of the *R. leguminosarum* symbiosis. *Penicillium bilaii* is registered under the Canadian Fertilizers Act for use with wheat, canola, pea, and lentil under the trade name PROVIDE™.

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